Journal of the American Mosquito Control Association, 12(1):52–57, 1996

OBSERVATIONS ON NOCTURNAL BITING ACTIVITY AND HOST PREFERENCE OF ANOPHELINES COLLECTED IN SOUTHERN THAILAND[1]

RAMPA RATTANARITHIKUL,[2,3] EIJI KONISHI[3] AND KENNETH J. LINTHICUM[2]

ABSTRACT. Over a 13-month period, 5,127 adult female mosquitoes were collected on human bait during multiple collecting periods between 1800 and 0600 h in 5 villages in southern Thailand. There were marked differences in the biting activity of different species at each of the villages studied. *Anopheles maculatus* and *Anopheles sawadwongporni* were collected most often at dusk or in the first hours of darkness between 1800 and 2100 h. Although specimens of *Anopheles dirus* were collected consistently between 1900 and 0400 h, peak collections were made between 2000 and 2300 h. *Anopheles minimus* was collected consistently throughout the night without a clearly discernible peak. Of more than 2,400 *Anopheles* mosquitoes collected in CDC light traps, 133 specimens (5.4%) contained blood, nearly all of which was identified by enzyme-linked immunosorbent assay (ELISA) to be of bovine origin. Ten specimens contained blood from more than one host species.

INTRODUCTION

Although *Anopheles dirus* Peyton and Harrison and *Anopheles minimus* Theobald are considered to be the principal vectors of malaria throughout much of Thailand (Rosenberg et al. 1990), little is known about the ecology of anopheline malaria vectors in peninsular Thailand. This area is particularly important ecologically because it serves as a crossroads for faunal dispersal from the Indian, Chinese, and Malaysian–Indonesian geographic regions (Harrison 1980). Knowledge of the biting habits of anopheline mosquitoes associated with malarious areas is of importance in establishing the vector potential of a species and in understanding the epidemiology of malaria. Furthermore, detailed information on the feeding and host-seeking behavior of mosquitoes can be used to improve the design of repellent or avoidance strategies and to improve malaria control projects. The purpose of this study was to determine the bloodfeeding activity patterns of anophelines seeking human blood and to identify mosquito host preference patterns in species found in an endemic malarious area of southern Thailand along the Myanmar border.

MATERIALS AND METHODS

Study area: Five collection sites were located in Thasalao, Palao-U, Wangpao, Salui, and Phato villages in Phetchaburi, Prachaup Khiri Khan, and Chumphon provinces in southern Thailand near the Myanmar border. Details concerning climate, geography, and human and animal populations at the collection sites are presented in an accompanying paper (Rattanarithikul et al. 1996b). At these collection sites, sunset and sunrise ranged from 1750 to 1850 h and 0555 to 0650 h, respectively, throughout the year (Technical Support Sub-Division, Forecast Division 1995).

Mosquito collections: To determine the time of bloodfeeding activity, man-biting collections were conducted outdoors 2–3 m away from a house for one or 2 nights per month over a 13-month period. A landing–biting collection for a night consisted of 12 cycles of 50 min collection and 10 min rest. A pair of collectors worked between 1800 and 2400 h and a second pair between 2400 and 0600 h. At the end of each 1-h collection cycle, mosquitoes collected in vials were placed in a paper cup covered with netting, labeled with the time of collection, and stored alive in cool boxes.

To determine host preference patterns, bloodfed mosquitoes were removed from light trap collections. These were made once or twice each month for 13 months using CO_2-baited CDC light traps. Light traps were placed overnight under trees (2 m above ground) 10–20 m from a dwelling. All *Anopheles* mosquitoes were identified to species using adult keys to anopheline mosquitoes in Thailand (Rattanarithikul and Green 1986, Rattanarithikul and Panthusiri 1994).

Blood meal identification: Host blood in engorged mosquitoes collected from light traps was identified using a single sandwich enzyme-linked immunosorbent assay (ELISA) (Konishi and Yamanishi 1984). All specimens that were thought to contain blood, based on their appear-

[1] The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

[2] Department of Entomology, U.S. Army Medical Component–Armed Forces Research Institute of Medical Sciences, AFRIMS, Rajvithi Road, Bangkok 10400 (within the USA use USAMC-AFRIMS, APO AP 96546).

[3] Department of Medical Zoology, Kobe University School of Medicine, Kobe 650, Japan.

Table 1. Nocturnal biting activity of *Anopheles* species collected at human bait in the village of Palao-U, Prachaup Khiri Khan Province, Thailand.

	Percentage of total number of specimens collected for each species						
Time (hour)	*An. dirus* (n = 31)	*An. maculatus* (n = 144)	*An. minimus* (n = 1,113)	*An. sawadwongporni* (n = 387)	*An. nivipes* (n = 3)	*An. barbirostris* gp.[1] (n = 121)	*An. hyrcanus* gp.[2] (n = 56)
1800	0	30.0	4.0	44.0	33.3	53.7	55.4
1900	6.5	37.5	10.8	22.5	66.7	16.5	8.9
2000	16.0	19.5	15.0	12.7	0	2.5	8.9
2100	22.5	4.3	11.1	8.5	0	4.1	3.6
2200	19.4	3.5	10.7	2.8	0	1.7	1.8
2300	9.7	1.3	9.2	0.5	0	2.5	0
2400	3.2	1.3	6.6	2.1	0	1.7	0
0100	9.7	1.3	11.7	0.5	0	2.5	1.8
0200	6.5	0	7.4	1.0	0	3.3	3.6
0300	6.5	0	4.5	0.8	0	1.7	0
0400	0	0	3.4	0.5	0	0.8	7.1
0500	0	1.3	5.6	4.1	0	9.0	8.9

[1] Includes *An. barbirostris* and *An. campestris.*
[2] Includes *An. nigerrimus, An. nitidus, An. peditaeniatus,* and *A. sinensis.*

ance, were triturated in a microvial. Specimens that had no detectable color of blood after trituration were not tested. Whole bodies of mosquitoes were triturated in 1 ml of ELISA diluent (phosphate-buffered saline containing 5% horse serum and 0.05% Tween 20). Antisera used as capture antibodies included goat anti-cat IgG, rabbit anti-bovine IgG, rabbit anti-chicken IgG, rabbit anti-human IgG (Cappel Laboratories Co. Ltd., Durham, NC), rabbit anti-dog IgG (Miles-Yeda, Ltd., Rehovot, Israel), and rabbit anti-porcine IgG (Yagai Co. Ltd., Tokyo). The IgG fractions of these antisera, except the rabbit anti-human IgG, were conjugated to horseradish peroxidase (Sigma Chemical Co.) by the method described by Wilson and Nakane (1978) to prepare detection antibodies. The horseradish peroxidase conjugate of anti-human IgG was purchased from Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD. Positive and negative controls were prepared as a 1:100 dilution of one homologous serum and 4 heterologous sera, respectively. Microtiter plates were sensitized with the capture antibody, blocked with bovine serum albumin, incubated with the test sample, followed by the detection antibody, and visualized with o-phenylene diamine in the presence of hydrogen peroxide. Samples that exhibited absorbance values at 495 nm greater than 3 SD above the mean of the negative controls were considered positive.

RESULTS

We collected 5,127 adult female *Anopheles* mosquitoes, consisting of 17 species, on human bait. The nocturnal biting activity of the 7 most common anopheline species collected at human bait in Palao-U is shown in Table 1. *Anopheles maculatus* Theobald and *Anopheles sawadwongporni* Rattanarithikul and Green were collected more often between 1800 and 2000 h than during all other time periods (chi-square, $P < 0.01$). Although *An. dirus* was collected between 1900 and 0300 h, peak collections were made between 2000 and 2300 h. Biting activity during this period was higher than during other periods (chi-square, $P < 0.01$). *Anopheles minimus* was collected throughout the night without a clearly discernible peak; however, more specimens were collected during the first half of the night (1800–2300 h) than during the second half of the night (2400–0500 h) (chi-square, $P < 0.01$). All *Anopheles nivipes* (Theobald) and the majority of *Anopheles barbirostris* Van der Wulp and *Anopheles campestris* Reid (here referred to as the *An. barbirostris* group) and *Anopheles crawfordi* Reid, *Anopheles nigrerrimus* Giles, *Anopheles nitidus* Harrison, Scanlon, and Reid, *Anopheles pediataeniatus* (Leicester), and *Anopheles sinensis* Wiedemann (here referred to as the *An. hyrcanus* group) were collected before 2000 h.

There were no significant differences between months in the time of peak feeding for any spe-

Table 2. Nocturnal biting activity of 4 potential *Anopheles* malaria vectors at human bait in Thasalao (T), Wangpao (W), Salui (S), and Phato (P) villages in southern Thailand.

	Percentage of total number of specimens collected for each species							
	An. dirus				*An. maculatus*			
Time (hour)	T (n = 20)	W (n = 0)	S (n = 5)	P (n = 28)	T (n = 1)	W (n = 50)	S (n = 35)	P (n = 106)
1800	10.0	0	0	7.1	0	14.0	2.9	9.4
1900	0	0	0	3.6	0	20.0	34.2	32.1
2000	10.0	0	0	14.3	0	26.0	20.0	27.4
2100	10.0	0	0	25.0	0	24.0	22.9	5.7
2200	50.0	0	40.0	17.8	0	12.0	14.2	5.7
2300	20.0	0	20.0	14.3	100.0	2.0	2.9	0.9
2400	0	0	0	10.7	0	0	2.9	0.9
0100	0	0	0	3.6	0	0	0	3.8
0200	0	0	40.0	3.6	0	0	0	1.9
0300	0	0	0	0	0	0	0	2.8
0400	0	0	0	0	0	0	0	1.9
0500	0	0	0	0	0	2.0	0	7.5

cies in Palao-U except for *An. minimus*. During May there were significantly more *An. minimus* collected after 2400 h than before (chi-square, $P < 0.01$).

The nocturnal biting activity of 4 potential malaria vectors at Wangpao, Salui, Phato, and Thasalao villages in southern Thailand is shown in Table 2. In general, the results are similar to observations in Palao-U with a few exceptions. More *An. maculatus* were found biting between 1800 and 1900 h in Palao-U than in each of the other villages. More *An. dirus, An. maculatus,* and *An. minimus* were collected after 2400 h in Phato than in Wangpao, Salui, and Thasalao.

Of 2,489 female mosquitoes collected in light traps, 133 (5.3%) specimens composed of 11 anopheline species had evidence of blood in the abdomen. The blood meals of all 11 species were identified (Table 3) and more than 96% contained bovine blood. Six specimens (4.5% of the total tested) of *Anopheles aconitus* Doenitz, *An. minimus* and *An. barbirostris* group contained human blood. Ten specimens (7.5%), composed of *An. aconitus, An. minimus,* and *An. barbirostris* group, contained blood from more than one species of vertebrate host.

DISCUSSION

The time of peak biting in the village of Palao-U for *An. dirus* is similar to that reported for specimens collected (as *Anopheles balabacensis* Baisas) in central Thailand (Wilkinson et al. 1970) but is in contrast to reports from southeast Thailand that described the peak feeding activity either between 2400 and 0300 h (Scanlon and Sandhinand 1965) or between 2200 and 0100 h (Rosenberg et al. 1990). Because *An. dirus* comprises a species complex involving at least 5 distinct species in Thailand that can be differentiated only by using either cytogenetic (Baimai et al. 1988) or DNA probe (Panyim et al. 1988) techniques, it is possible that the species studied in these reports may represent different taxa. Baimai et al. (1988) reported that *An. dirus* species A, B, C, and D each have distinct times of biting during the night. The biting activity pattern we observed was not consistent with that of any of the *An. dirus* species reported by Baimai et al. (1988). We did not confirm the identity of the *An. dirus* collected; however, species A, C, and D have been found within the geographic range of the present study (Baimai 1988).

In Palao-U, more than 85% of all *An. maculatus* were collected before 2100 h. This early biting activity pattern was similar to that previously reported elsewhere in Thailand (Scanlon and Sandhinand 1965, Wilkinson et al. 1970, Harbach et al. 1987). Also, similar to what has been observed elsewhere in Thailand (Scanlon and Sandhinand 1965, Harbach et al. 1987), *An. minimus* fed throughout the night without a distinct peak.

We observed that in Palao-U villagers hunt and trap in the forest after sunset and that families remain active in the village until at least 2100 h. The early evening biting activity observed for *An. dirus* and *An. maculatus* in each of the villages in peninsular Thailand suggests that there would be considerable human exposure to these species before bed nets would normally serve as a barrier to biting. Conversely, the extended biting activity of *An. minimus* suggests that the use of bed nets may reduce ex-

Table 2. Extended

Percentage of total number of specimens collected for each species							
An. minimus				*An. sawadwongporni*			
T (n = 74)	W (n = 67)	S (n = 534)	P (n = 1,977)	T (n = 5)	W (n = 12)	S (n = 1)	P (n = 2)
1.4	13.4	2.3	0.2	0	16.7	0	0
5.4	14.9	10.7	4.5	20.0	8.3	100.0	100.0
13.5	10.5	11.8	7.6	20.0	16.7	0	0
18.9	9.0	17.0	7.0	0	16.7	0	0
36.5	21.0	12.8	10.7	40.0	25.0	0	0
24.3	17.9	10.9	10.7	20.0	8.3	0	0
0	3.0	4.9	8.5	0	0	0	0
0	3.0	6.9	13.8	0	0	0	0
0	1.3	6.2	11.6	0	0	0	0
0	3.0	4.9	8.9	0	0	0	0
0	0	2.8	5.9	0	0	0	0
0	3.0	8.8	10.6	0	8.3	0	0

posure to more than 70% of host-seeking mosquitoes of this species. Rattanarithikul et al. (1996a) previously found that the head and thorax of 1.4% of *An. dirus,* 0.7% of *An. maculatus,* and 0.3% of *An. minimus* collected in peninsular Thailand contained *Plasmodium falciparum* circumsporozoite (CS) antigen. In Palao-U, *An. sawadwongporni, An. nivipes, An. barbirostris* group, and *An. hyrcanus* group, which all exhibited early biting activity peaks, were also found to contain *P. falciparum* CS protein (Rattanarithikul et al. 1996a). The detection of CS antigen in the head and thorax indicates the presence of sporozoites in the hemolymph, suggesting possible infection of the salivary glands.

All specimens tested for blood meal analysis were collected with light traps. This method probably introduced a bias toward exophilic species. It is important to realize that bloodfed specimens represented only about 5% of the total specimens collected in light traps. No bloodfed *An. dirus* were found.

More than 96% of specimens containing blood had fed on cattle, although light traps were placed 10–20 m away from cattle. Furthermore, humans and dogs were more numer-

Table 3. Bloodfeeding patterns of 11 *Anopheles* species collected in CDC light traps in southern Thailand.

Species	No. mosquitoes with blood in abdomen							
	Human blood	Cattle blood	Pig blood	Human/ dog blood	Human/ cattle blood	Cattle/ pig blood	Cattle/ dog blood	Cattle/ dog/cat blood
An. aconitus	—	13	1	—	2	2	—	—
An. culicifacies	—	1	—	—	—	—	—	—
An. jamesii	—	7	1	—	—	—	—	—
An. kochi	—	1	—	—	—	—	—	—
An. maculatus	—	3	—	—	—	—	—	—
An. minimus	2	46	—	1	1	—	1	1
An. sawadwongporni	—	3	—	—	—	—	—	—
An. tessellatus	—	3	—	—	—	—	—	—
An. varuna	—	1	—	—	—	—	—	—
An. barbirostris gp.[1]	—	29	—	—	1	1	—	—
An. hyrcanus gp.[2]	—	12	—	—	—	—	—	—
Total	2	119	2	1	4	3	1	1

[1] Includes *An. barbirostris* and *An. campestris.*
[2] Includes *An. nigerrimus, An. nitidus, An. peditaeniatus,* and *An. sinensis.*

ous in the villages than were cattle. In resting collections in India, Roy et al. (1991) reported that the major species of *Anopheles* fed predominantly on cattle. It is possible that a higher percentage of bovine feeders are attracted to light. However, in studies in which specimens were collected in light traps placed adjacent to areas where cattle were the predominant large vertebrate, the proportion of mosquitoes of various genera and species containing bovine blood never exceeded 85% (Linthicum et al. 1985, Gordon et al. 1991).

Anopheles minimus accounted for the most bloodfed specimens. Bovine blood meals were most numerous irrespective of collection site. *Anopheles minimus* also accounted for the greatest number of human blood meals. The only other human blood meals were found in *An. aconitus* and *An. barbirostris* group. *Anopheles aconitus* is a rice field-breeding mosquito and is an important vector of malaria in southwestern Asia, although it is not considered to be an important vector in Thailand (Rao 1984).

Multiple mosquito feeding has been reported previously (Edman and Downe 1964, Boreham and Garrett-Jones 1973). This is an important factor involved in the transmission of infectious agents. Although our present study indicated that the rate of multiple feeding ranged from 7.7% in *An. minimus* to 22% in *An. aconitus,* bloodfed specimens represented less than 6.0% of all specimens collected in light traps. Edman and Downe (1964) reported the incidence of multiple feeding ranging from 9.7 to 61.8% in culicine species in Kansas. With the ELISA test used in the present study, multiple feeding was detected only when mosquitoes fed on different species of animals. Actual multiple feeding rates are probably higher than test results indicate because multiple blood meals may occur on the same species of host (e.g., human–human). Estimates of multiple bloodfeeding using histological techniques have been developed to detect multiple blood meals during a single gonotrophic cycle from the same species of host (Romoser et al. 1989). Studies in Thailand using these techniques found that most *Aedes aegypti* (Linn.) collected had fed twice in each gonotrophic cycle (Scott et al. 1993). Species with a greater likelihood of taking multiple blood meals may have a higher vectorial capacity by increasing the chances of acquiring and transmitting a disease agent (Boreham and Garrett-Jones 1973). It is also possible that multiple feeding reduces the potential of a mosquito to serve as an efficient malaria vector by reducing the likelihood of obtaining enough gametocytes to become infected and by diminishing the number of sporozoites inoculated (Burkot et al. 1988).

ACKNOWLEDGMENTS

We thank Ronald Rosenberg, Takeo Matsumura, and Takeshi Kurihara for support and criticism of the study; Chumnong Noikamol, Somporn Chanaimongkol, Pradith Mahaphibul, and Suda Rattanawong for field and laboratory assistance; and Somprathana Kiewlongya, Prachong Panthusiri, and Scott W. Gordon for assistance in preparation of the manuscript. This study was supported in part by the Japan Society for Promotion of Science Ronpaku Program.

REFERENCES CITED

Baimai, V. 1988. Population cytogenetics of the malaria vector *Anopheles leucosphyrus* group. Southeast Asian J. Trop. Med. Public Health 19:667–680.

Baimai, V., U. Kijchalao, P. Sawadwongporn and C. A. Green. 1988. Geographic distribution and biting behaviour of four species of the *Anopheles dirus* complex (Diptera: Culicidae) in Thailand. Southeast Asian J. Trop. Med. Public Health 19:151–161.

Boreham, P. F. L. and C. Garrett-Jones. 1973. Prevalence of mixed blood meals and double feeding in a malaria vector (*Anopheles sacharovi* Favre). Bull. W.H.O. 48:605–614.

Burkot, T. R., P. M. Graves, R. Paru and M. Lagog. 1988. Mixed blood feeding by the malaria vectors in the *Anopheles punctulatus* complex (Diptera: Culicidae). J. Med. Entomol. 25:205–213.

Edman, J. D. and A. E. R. Downe. 1964. Host-blood sources and multiple-feeding habits of mosquitoes in Kansas. Mosq. News 24:154–160.

Gordon, S. W., R. F. Tammariello, K. J. Linthicum, R. A. Wirtz and J. P. Digoutte. 1991. Feeding patterns of mosquitoes collected in the Senegal River basin. J. Am. Mosq. Control Assoc. 7:424–432.

Harbach, R. E., J. B. Gingrich and L. W. Pang. 1987. Some entomological observations on malaria transmission in a remote village in northwestern Thailand. J. Am. Mosq. Control Assoc. 3:296–301.

Harrison, B. A. 1980. Medical entomology studies—XIII. The Myzomyia series of *Anopheles* (*Cellia*) in Thailand, with emphasis on intra–interspecific variations (Diptera: Culicidae). Contrib. Am. Entomol. Inst. (Ann Arbor) 17(4):1–195.

Konishi, E. and H. Yamanishi. 1984. Estimation of blood meal size of *Aedes albopictus* (Diptera: Culicidae) using enzyme-linked immunosorbent assay. J. Med. Entomol. 21:506–513.

Linthicum, K. J., H. G. A. Kaburia, F. G. Davies and K. J. Lindqvist. 1985. A blood meal analysis of engorged mosquitoes found in Rift Valley fever epizootics areas in Kenya. J. Am. Mosq. Control Assoc. 1:93–95.

Panyim, S., S. Yasothornsrikul and V. Baimai. 1988. Species-specific DNA sequences from the *Anopheles dirus* complex—a potential for efficient identifications of isomorphic species, pp. 193–202. *In:* M. W. Service. (ed.). Biosystematics of haematophagus insects. Syst. Assoc. Spec. Vol. 37.

Rao, T. R. 1984. The *Anopheles* of India. Malaria Research Centre, Indian Council of Medical Research, New Delhi.

Rattanarithikul, R. and C. A. Green. 1986. Formal recognition of the species of the *Anopheles maculatus* group (Diptera: Culicidae) occurring in Thailand, including the descriptions of two new species and a preliminary key to females. Mosq. Syst. 18: 246–278.

Rattanarithikul, R. and P. Panthusiri. 1994. Illustrated keys to the medically important mosquitoes of Thailand. Southeast Asian J. Trop. Med. Public Health 25(Suppl.):1–66.

Rattanarithikul, R., E. Konishi and K. J. Linthicum. 1996a. Detection of *Plasmodium vivax* and *Plasmodium falciparum* circumsporozoite antigen in anopheline mosquitoes collected in southern Thailand. Am. J. Trop. Med. Hyg. (in press)

Rattanarithikul, R., K. J. Linthicum and E. Konishi. 1996b. Seasonal abundance and parity rates of *Anopheles* species in southern Thailand. J. Am. Mosq. Control Assoc. 12:75–83.

Romoser, W. S., J. D. Edman, L. H. Lorenz and T. W. Scott. 1989. Histological parameters useful in the identification of multiple bloodmeals in mosquitoes. Am. J. Trop. Med. Hyg. 41:737–742.

Rosenberg, R., R. G. Andre and L. Somchit. 1990. Highly efficient dry season transmission of malaria in Thailand. Trans. R. Soc. Trop. Med. Hyg. 84:22–28.

Roy, A., M. A. Ansari and V. P. Sharma. 1991. Feeding behavior patterns of anophelines from Uttar Pradesh and Gujarat states of India. J. Am. Mosq. Control Assoc. 7:11–15.

Scanlon, J. E. and J. Sandhinand. 1965. The distribution and biology of *Anopheles balabacensis* in Thailand (Diptera: Culicidae). J. Med. Entomol. 2: 61–69.

Scott, T. W., G. G. Clark, L. H. Lorenz, P. H. Amerasinghe, P. Reiter and J. D. Edman. 1993. Detection of multiple blood feeding in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using a histologic technique. J. Med. Entomol. 30:94–99.

Technical Support Sub-Division, Forecast Division. 1995. Weather almanac. Thailand Meteorological Department, Bangkok, Thailand.

Wilkinson, R. N., T. A. Miller and S. Esah. 1970. Anthropophilic mosquitoes in central Thailand, with notes on *Anopheles balabacensis* Baisas and malaria. Mosq. News 30:146–148.

Wilson, M. B. and P. K. Nakane. 1978. Recent developments in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies, pp. 215–224. *In:* W. Knapp, K. Holular and G. Wick (eds.). Immunofluorescence and related staining techniques. Elsevier/North Holland Biomedical Press, Amsterdam.